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# Hexavalent Chromate Reduction During Growth and by Immobilized Cells of *Arthrobacter* sp. SUK 1205

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**Abstract:** The chromate reducing actinomycetes, *Arthrobacter* sp. SUK 1205, isolated from chromite mine overburden of Odisha, India exhibited significant chromate reduction during growth with characteristic formation of pale green insoluble precipitate. Reduction of chromate increased with increase in inoculum density but the reduction potential declined as and when Cr(VI) concentration in the medium was increased. Chromate reducing efficiency was promoted when glycerol and glucose were used as electron donors and pH and temperature were maintained at 7.0 and 35°C, respectively. The reduction process was inhibited by several metal ions and metabolic inhibitors but not by Cu(II), Mn(II) and DNP. Among the matrices tested for whole cell immobilization, Ca-alginate immobilized whole cells were found to be most effective and were comparable with non-immobilized cells. Minimal salts (MS) medium was the most effective base for Cr(VI) reduction studies with immobilized cells. Under such conditions, the immobilized cells retained their enzymatic activity at least for 4 consecutive cycles indicating the potential of *Arthrobacter* sp. SUK 1205 in bioremediation of environmental chromium pollution.

**Key words:** *Arthrobacter*, chromite mine overburden, Cr(VI) reduction, chromium bioremediation, immobilized cells, Ca-alginate

## INTRODUCTION

Actinomycetes represent a significant constituent of the microbial population in most of natural and man made environments and are extensively distributed in soil, marine sediments, heavy metal polluted soils, plants and animals (You *et al*., 2005). The group is characterized by specific growth patterns, rapid colonization of selective substrates and wide metabolic diversities showing production of as well as resistance for antimicrobial molecules (Nodwell, 2007; Jain *et al*., 2012). Heavy metal resistance and reducing capability of this unique group of microorganisms in stressed environments have identified them as suitable agents for bioremediation (Polti *et al*., 2010).

Chromium is an essential micronutrient for living organisms. It exists in nine valence states but in nature mainly occurs as Cr(VI) in the divalent oxyanion chromate form and Cr(III) as trivalent cation because of their stability. Cr(VI) is toxic and has a stronger oxidizing power and higher membrane transport capability (Katz and Salem, 1994). The toxicity of Cr(VI) is mainly attributed to the process of reduction of Cr(VI) to lower oxidation state, resulting in the production of free radicals, which leads to oxidative stress, DNA damage and ultimately altered gene expression. Due to these harmful and deleterious effects, Cr(VI) has been listed as a priority pollutant and classified as a class A human carcinogen by the US Environmental Protection Agency (USEPA) (Costa and Klein, 2006).

Microbiological reduction of Cr(VI) to and its precipitation into immobile Cr(III) by chromium resistant and reducing bacteria is considered to be an effective method for detoxification of Cr(VI)-contaminated environments and have a potential use in bioremediation (Cheung and Gu, 2003, 2007). Members of actinomycetes are no exception to this. The first report on Cr(VI) reduction by *Streptomyces* was documented by Das and Chandra (1990). Subsequently, Laxman and More (2002), Mabrouk (2008) and Polti *et al*. (2009) determined Cr(VI) reduction by *Streptomyces griseus*, *Streptomyces* MS-2 and *Streptomyces* sp. MC1 respectively. Bacterial isolates belonging to *Aureobacterium*, *Clavibacter* (Francisco *et al*., 2002), *Cellulomonas* sp. (Sani *et al*.,

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2002), *Microbacterium* (Pattanapipitpaisal *et al*., 2001; Liu *et al*., 2012) and *Arthrobacter* sp. (Camargo *et al*., 2004; Asatiani *et al*., 2004; Quintelas *et al*., 2007; Elangovan *et al*., 2010; Dey and Paul, 2012a, b) have also been studied for their Cr(VI) reducing capacity.

The chromate reducing ability of the members of the genus *Arthrobacter* has been explored by several authors (Megharaj *et al*., 2003; Asianti *et al*., 2004; Camargo *et al*., 2004; Horton *et al*., 2006; Dey and Paul, 2012a, b), using viable whole cells as well as by cell-free extracts (Elangovan *et al*., 2010; Dey and Paul, 2012b, 2013b). *Arthrobacter* species, immobilized in different matrices such as Ca-alginate (Elangovan *et al*., 2010) and Ba-alginate (Dey and Paul, 2014) have also been documented for their Cr(VI) reducing ability. Cr(VI) reduction with immobilized cells of *Bacillus* sp. (Camargo *et al*., 2004), *Intrasporangium* sp. Q5-1 (Yang *et al*., 2009), *Cellulosimicrobium cellulans* KUCr3 (Chatterjee *et al*., 2011) and *Pannonibacter phragmitetus* LSSE-09 (Xu *et al*., 2011) has also attracted the attention for effective exploitation.

An *Arthrobacter* strain SUK 1205 (MTCC accession No. 8731 and NCBI GenBank Accession No. JQ312666) showing 98% similarity with *Arthrobacter* sp. 3-4A and having a profound chromate reducing ability has been reported from this laboratory (Dey and Paul, 2012b, 2013a). This study reports the optimization of conditions for Cr(VI) reduction during growth of *Arthrobacter* sp. SUK 1205, immobilization of SUK 1205 whole cells for Cr(VI) reduction and recycling of immobilized cells for long term use.

## MATERIALS AND METHODS

**Source and maintenance of bacterial isolate:** Chromate reducing bacterium *Arthrobacter* SUK 1205 (MTCC 8731, GenBank Accession No. JQ312666) isolated from chromite mine overburden of Odisha, India was used throughout the present study. The bacterium was grown and maintained on slopes of Peptone Yeast-Extract and Glucose (PYEG) agar medium (Wang and Xiao, 1995) containing (g $L^{-1}$) peptone, 10.0; yeast extract, 5.0; glucose, 3.0 and agar, 20.0 (pH 7.0), supplemented with 2 mM Cr(VI).

**Reduction of Cr(VI) during growth:** Chromium reduction studies during growth of *Arthrobacter* sp. SUK 1205 were conducted in Vogel Bonner (V. B.) broth. The V. B. broth contained 2% of sterile stock solution of V. B. concentrate containing (g $L^{-1}$): anhydrous $K_2HPO_4$, 500.0; $Na(NH_4)HPO_4.4H_2O$, 175.0; citric acid, 100.0; $MgSO_4.7H_2O$, 10.0 and 2.0% of 25% D-glucose 20.0 mL (pH 7.0) in 1 L distilled water (Wang and Xiao, 1995). The medium (25 mL/100 mL flask) was inoculated with overnight grown cultures in PYEG medium and incubated at 35°C under continuous shaking (120 rpm). Unless otherwise mentioned, the initial inoculum was maintained at $10^6$ cells $mL^{-1}$ in all experiments. In order to monitor any abiotic Cr(VI) reduction, cell-free controls were used. The growth of the isolate was determined by viable cell count method, while the residual hexavalent chromium was measured following the usual diphenyl carbazide method (Park *et al*., 2000).

As and when required, total chromium in the culture filtrate was measured using varian atomic absorption spectrophotometer (SpectrAA-20Plus). Total protein was estimated by Folin-phenol reagent (Lowry *et al*., 1951) and glucose was quantified with 3,5-dinitrosalicylic acid reagent (Bernfeld, 1955).

**Immobilization of cells:** For immobilization of *Arthrobacter* sp. SUK 1205 cells sodium alginate and polyvinyl alcohol (Av. Mol wt. 70,000-1,00,000) were used as matrix. Freshly harvested, washed cell suspension was added to the cooled sterilized alginate (2%,w/v) solution, mixed well and the mixture was extruded drop by drop into cold sterile 0.2 M $BaCl_2$ and $CaCl_2$ solutions through sterile 5 mL pipette. The beads thus formed were hardened in 0.2 M $BaCl_2$ or $CaCl_2$ solution for 24 h at 4°C. Finally, the beads were thoroughly washed with sterile distilled water before use (Johnsen and Flink, 1986). Similarly, cells were also immobilized in PVA-Na-alginate mixture following extrusion of cell suspension either in mixture of saturated boric acid and 2% $CaCl_2$, $2H_2O$ (Pattanapipitpaisal *et al*., 2001) or only in 2% $CaCl_2$, $2H_2O$. The beads were further strengthened in the same solutions for overnight at 4°C (Wu and Wisecarver, 1992), washed with sterile distilled water and used for chromate reduction studies.

**Chromium reduction by immobilized cell beads:** Chromate reducing activity of immobilized cell beads were evaluated in batch culture using Mineral Salts (MS) medium (Wang and Xiao, 1995), Tris buffer supplemented with 0.1% glucose, Tris buffer, Peptone Yeast Extract Glucose medium (PYEG) and V. B. broth supplemented with 100 µM Cr(VI). Beads containing bacterial cells equivalent to $10^9$ cells $mL^{-1}$ were added to the medium (15 mL/100 mL flask) and incubated at 35°C

in a rotary shaker (120 rpm). Samples were withdrawn aseptically at regular interval and assayed for residual Cr(VI) following the method of Park *et al*. (2000).

**Statistical analysis:** All experiments were carried out in triplicate and results represent Mean±Standard Error.

## RESULTS

**Chromate reduction during growth:** During growth in V.B. broth supplemented with 2 mM Cr(VI), *Arthrobacter* sp. SUK 1205 showed gradual discoloration of the medium and formation of a pale green precipitates along with decrease in 64% of the Cr(VI) content. During the course of hexavalent chromium reduction in culture, there was a gradual increase in cell count till 8th day of incubation, which was nearly 9.06±0.12 log No. of cells $mL^{-1}$ (Fig. 1). A gradual increase in the total protein from 0.2±0.1-0.65±0.09 mg $mg^{-1}$ of cell dry wt was also noticed and was accompanied with the gradual decrease in the glucose content of the medium.

**Effect of initial Cr(VI) concentration:** The influence of Cr(VI) concentration on its reduction by SUK 1205 was tested in the range of 0.5-6.0 mM (Fig. 2). Complete reduction of hexavalent chromium in the medium occurred within 5 days of incubation at the lowest concentration (0.5 mM). However, with the increase in chromium concentration (1-6 mM), complete reduction of Cr(VI) could not be achieved although an increase in log no. of cells $mL^{-1}$ was evident at these higher concentrations.

**Effect of inoculum density:** Effect of inoculum density of the selected bacterial isolate was tested on the growth and chromate reduction under shake conditions. Freshly prepared inoculum was added to the reduction medium at a cell density ranging from $10^5$-$10^{10}$ cells $mL^{-1}$ and Cr(VI) reduction study was conducted under batch culture (Fig. 3). It was evident that with increase in cell density, the reduction potential of SUK 1205 gradually increased. The optimum cell density was found to be $10^{10}$ cells $mL^{-1}$ where nearly 90% of initial 2 mM Cr(VI) was reduced by the isolate. At the highest cell concentration tested ($10^{10}$cells $mL^{-1}$), very little cell growth was observed. At lower cell concentration ($10^5$ cells $mL^{-1}$) only 56% Cr(VI) was reduced (Fig. 3).

**Effect of electron donor:** Reduction of Cr(VI) during growth of *Arthrobacter* sp. SUK 1205 was studied in presence of propionate, acetate, benzoate, fumarate, glucose, sucrose, glycerol, propylene glycol, chlorophenol, glycine, yeast extract, tryptone and cresol as electron donors (Table 1). Glycerol as the electron donor completely reduced of initial 2 mM chromate during 8 days of incubation. This was followed by

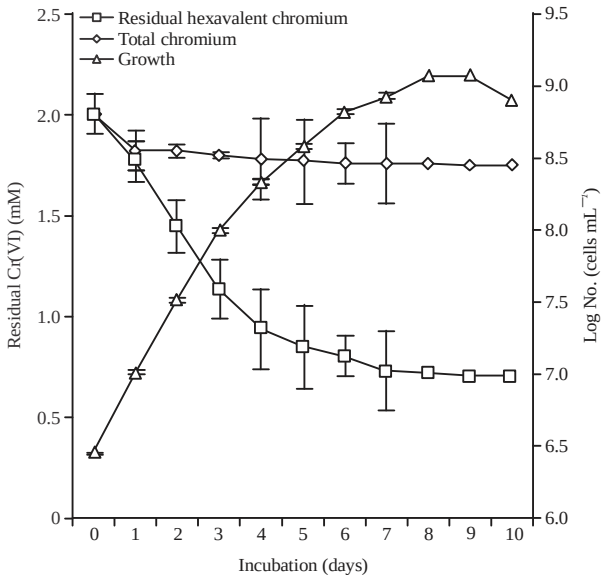


Fig. 1: Growth and Reduction of hexavalent chromium by *Arthrobacter* sp. SUK 1205 in Vogel Bonner broth under batch culture

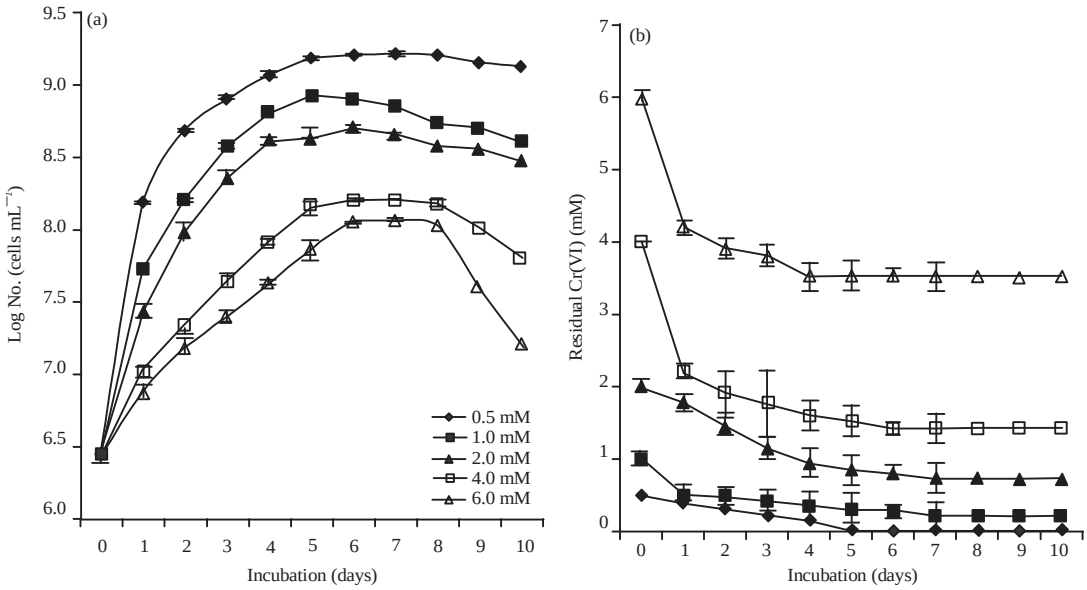


Fig. 2(a-b): Effect of concentration of Cr(VI) on (a) Growth and (b) Cr(VI) reduction by *Arthrobacter* sp. SUK 1205

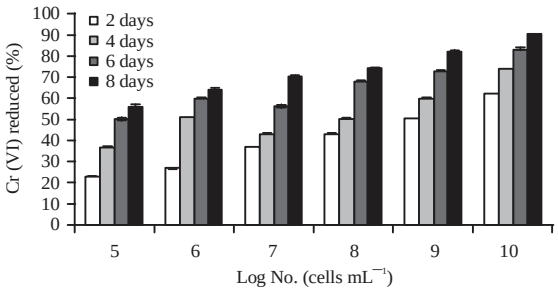


Fig. 3: Effect of inoculum density on Cr(VI) reduction by *Arthrobacter* sp. SUK 1205

glucose where 73% of initial Cr(VI) was reduced. In addition, the isolate was able to utilize a wide variety of electron donors including sugar, sugar alcohols, amino acids and organic acids with considerable variation of reduction efficiency. Yeast extract and propionate were least efficient as electron donors for SUK 1205 showing only 21.2 and 17.4% chromate reduction, respectively.

**Effect of temperature and pH:** The chromate reduction efficiency of the isolate SUK 1205 was much sensitive to the incubation temperature tested over a range of 25-40°C (Fig. 4a). Maximum (64.5%) chromate reduction was recorded at 35°C. While, the process was severely affected at 40°C which showed nearly 20% Cr(VI) reduction.

Similarly, chromate reduction by *Arthrobacter* sp. SUK 1205 appeared to be greatly influenced by the initial pH of the growth medium, maximum (64%) being observed at pH 7.0 (Fig. 4b).

**Effect of metal ions:** Addition of metal ions like Ni(II), Co(II), Zn(II) and Cd(II) in the growth medium in general resulted in inhibition of chromate reduction. However, presence of Cu(II) and Mn(II) was found to promote chromate reduction showing about 83 and 80% chromate reduction respectively as against 64% reduction of 2 mM Cr(VI) in the control set without additional metal supplementation. Cd(II) followed by Zn(II), Ni(II) and Co(II) (Fig. 5) appeared to be most toxic and thereby, impaired growth as well as chromate reduction by SUK 1205.

**Effect of inhibitor:** Remarkable inhibition of Cr(VI) reduction during growth of SUK 1205 was noticed with most of the inhibitors except DNP. It appeared neither

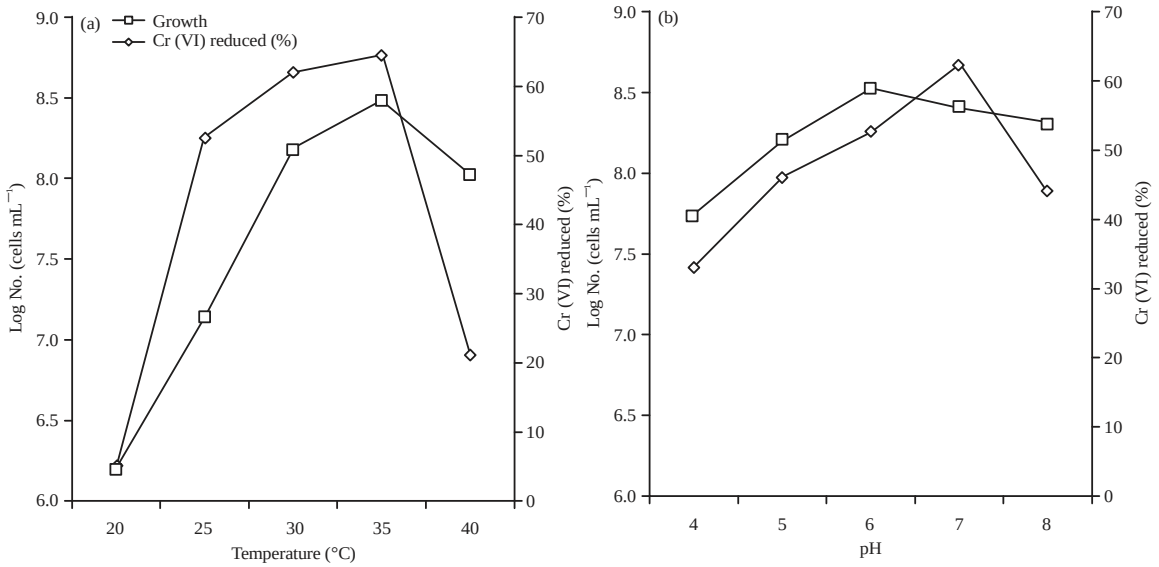


Fig. 4(a-b): Effect of (a) Temperature and (b) pH on growth and chromate reduction during growth by *Arthrobacter* sp. SUK 1205

Table 1: Effect of different electron donors on growth and Cr(VI) reduction by *Arthrobacter* sp. SUK 1205

| | Incubation (days) | | | | | |
|---|---|---|---|---|---|---|
| | 2 | | 4 | | 8 | |
| Electron donors[c] | a | b | a | b | a | b |
| Control | 7.80±0.06 | 36.5±0.1 | 8.5±0.01 | 48.0±0.6 | 8.60±0.00 | 64.3±0.1 |
| Glucose | 7.90±0.06 | 40.5±0.0 | 8.7±0.00 | 49.5±0.2 | 8.50±0.06 | 73.7±0.2 |
| Sucrose | 7.20±0.07 | 33.5±0.1 | 8.3±0.07 | 36.5±0.0 | 7.60±0.00 | 45.2±0.3 |
| Acetate | 6.80±0.03 | 20.5±0.2 | 7.7±0.00 | 21.0±0.6 | 7.50±0.06 | 24.7±0.0 |
| Propionate | 6.20±0.06 | 12.0±0.1 | 8.4±0.01 | 17.4±0.1 | 8.10±0.01 | 17.4±0.1 |
| Fumarate | 7.20±0.02 | 35.0±0.0 | 8.1±0.00 | 38.0±0.1 | 7.02±0.00 | 40.0±0.2 |
| Benzoate | 7.50±0.06 | 39.7±0.6 | 8.7±0.00 | 46.5±0.0 | 7.60±0.00 | 53.1±0.5 |
| Glycerol | 7.42±0.00 | 46.7±0.4 | 9.3±0.07 | 78.5±0.3 | 8.70±0.00 | 100.0±0.6 |
| O-chlorophenol | 6.80±0.03 | 16.5±0.1 | 7.7±0.00 | 50.8±0.1 | 8.30±0.07 | 53.1±0.0 |
| Propylene glycol | 6.30±0.02 | 10.0±0.0 | 7.3±0.07 | 36.5±0.2 | 7.02±0.00 | 56.0±0.0 |
| o-cresol | 6.80±0.03 | 9.7±0.3 | 8.1±0.01 | 26.5±0.1 | 7.90±0.00 | 26.5±0.0 |
| Glycine | 7.20±0.06 | 20.5±0.7 | 7.1±0.00 | 22.6±0.0 | 7.70±0.00 | 30.5±0.1 |
| Peptone | 6.80±0.03 | 22.0±0.2 | 7.7±0.00 | 24.0±0.0 | 7.10±0.00 | 23.5±0.4 |
| Yeast extract | 6.50±0.02 | 18.0±0.1 | 7.3±0.07 | 21.2±0.7 | 7.30±0.00 | 21.2±0.1 |
| Tryptone | 7.80±0.03 | 26.5±0.1 | 7.3±0.03 | 33.0±0.2 | 7.10±0.07 | 33.0±0.5 |

a: Growth, log no. of cells $mL^{-1}$, b: Cr(VI) reduced (%), [c]All electron donors were added to the chromate reduction medium at 0.1% (w/v) level, Results represent mean of triplicate sets±standard error

inhibitory nor promotive as it could reduce 62.5% of 2 mM Cr(VI) as against 64.3% in the control (Table 2). CCCP was most inhibitory followed by NaF showing 41 and 42% reduction of initial 2 mM Cr(VI), respectively.

**Effect of different media on chromate reduction by immobilized cells:** *Arthrobacter* sp. SUK 1205 cells immobilized in Ba-alginate, Ca-alginate, PVA-borate and PVA-alginate were tested for chromate reduction ability, bead integrity and leaching of cells from beads in five different media (Table 3). Experiments were carried out at 35°C under continuous shaking (120 rpm) in a rotary shaker. Results show that the combination of cells immobilized in Ca-alginate and Mineral Salts (MS) medium was the most effective combination for Cr(VI) reduction as complete reduction of 100 µM Cr(VI) occurred without any leakage of cells and visible disintegration of beads (Table 3). In half-strength

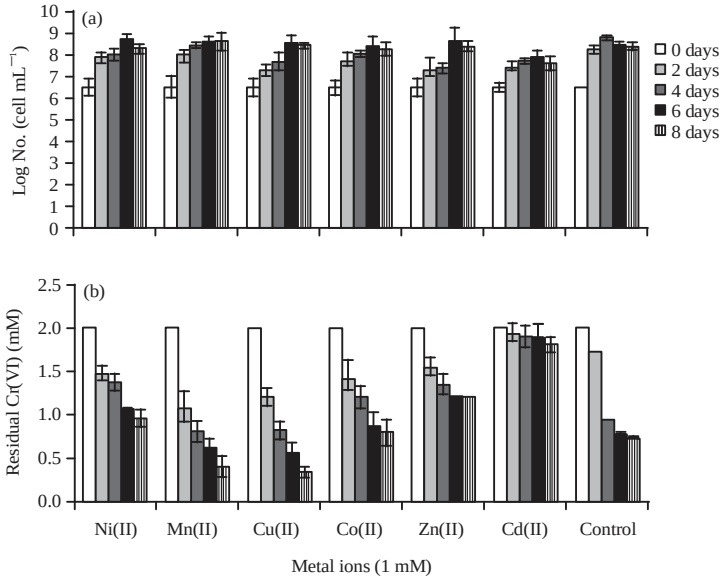


Fig. 5(a-b): Effect of additional metal ions on (a) Growth and (b) Cr(VI) reduction by *Arthrobacter* sp. SUK 1205

Table 2: Effect of different inhibitors on growth and Cr(VI) reduction by *Arthrobacter* sp. SUK 1205

| | Incubation (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | |
| Inhibitors[a] | b | c | b | c | b | c | b | c |
| Control (-Inhibitor) | 7.850±0.06 | 36.5±0.10 | 8.56±0.01 | 48.00±0.60 | 9.02±0.00 | 56.00±0.60 | 8.68±0.00 | 64.30±0.00 |
| CCCP | 7.024±0.02 | 23.5±0.22 | 7.12±0.00 | 38.50±0.00 | 7.06±0.00 | 40.00±0.02 | 7.02±0.00 | 41.00±0.02 |
| DCC | 7.236±0.04 | 46.7±0.12 | 7.55±0.02 | 53.10±0.06 | 7.26±0.06 | 54.60±0.00 | 7.12±0.02 | 54.60±0.00 |
| DNP | 7.602±0.04 | 53.1±0.34 | 7.44±0.00 | 59.00±0.02 | 7.38±0.00 | 60.50±0.06 | 7.25±0.02 | 62.50±0.06 |
| $NaN_3$ | 7.740±0.04 | 39.7±0.39 | 7.55±0.06 | 46.50±0.00 | 7.32±0.05 | 53.75± 0.00 | 7.08±0.00 | 53.75±0.00 |
| NaF | 7.124±0.02 | 29.5±0.07 | 7.12±0.06 | 42.00±0.00 | 7.01±0.00 | 42.00±0.06 | 6.80±0.00 | 42.00±0.00 |

[a]CCCP: Carbonyl cyanide-m-chlorophenyl hydrazone, DCC: N, N,-dicyclohexyl carbodiimide, DNP: 2,4-di nitrophenol, $NaN_3$: Sodium azide, NaF: Sodium fluoride. All inhibitors were added to the chromate reduction medium at 1 mM level, b: Growth, log no. of cells $mL^{-1}$; c: Cr(VI) reduced (%), Results represent mean of triplicate sets±standard error

Peptone Yeast Extract Glucose (PYEG) medium, both Ba-alginate and Ca-alginate immobilized cells showed Cr(VI) reduction comparable to these in MS medium but was associated with, significant leakage of cells. Tris buffer appeared to be a poor base for Cr(VI) reduction by immobilized cells but the efficiency was increased as and when it was supplemented with 0.1% glucose. While beads of all four different types were extremely unstable in V. B. broth and showed visible disintegration of beads.

**Time course of Cr(VI) reduction by immobilized cells:** A comparative study of Cr(VI) reduction was conducted in MS medium using viable immobilized cells of *Arthrobacter* sp. SUK 1205 in Ca-alginate, viable whole cells and immobilized heat killed cells (Fig. 6). Results show that chromate reducing efficiency of Ca-alginate immobilized cells and viable whole cells was more or less comparable and completely reduced 100 µM of Cr(VI) in the medium within 24 h of incubation. Heat killed cells immobilized in Ca alginate were unable to reduce Cr(VI) under identical conditions.

**Recycling of Ca-alginate beads:** Attempts were made to reuse the immobilizes cells and the recycling efficiency of Ca-alginate immobilized cells of *Arthrobacter* SUK 1205 was tested in MS medium containing 100 µM Cr(VI). The initial amount of chromium was completely reduced within 24 h during the first three consecutive cycles (Fig. 7). However, in the fourth cycle, the reduction efficiency of the immobilized cells declined showing about 90% Cr(VI) reduction.

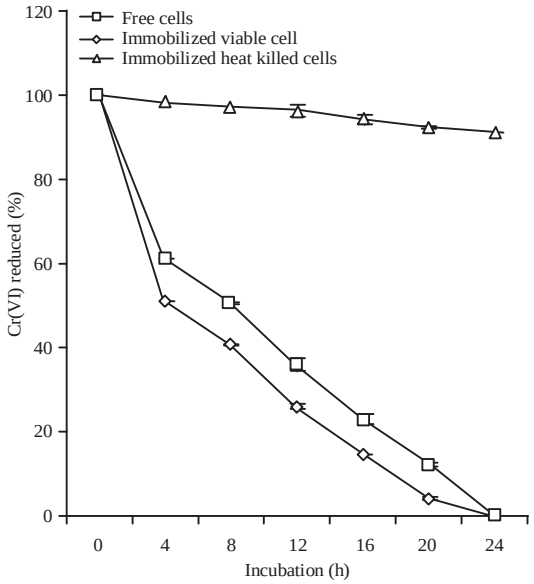


Fig. 6: Chromate reduction by free, immobilized and immobilized heat killed cells of *Arthrobacter* sp. SUK 1205

Table 3: Effect of different media on Cr(VI) reduction by whole cells of *Arthrobacter* sp. SUK 1205 immobilized in different matrices

| | Immobilization matrix | | | |
|---|---|---|---|---|
| Medium and parameter studied[a] | Ba-alginate | Ca-alginate | PVA-borate | PVA-alginate |
| **MS medium** | | | | |
| A | 76.85±2.2 | 100.0±0.0 | 49.27±1.7 | 44.64±0.6 |
| B | Retained | Retained | Retained | Retained |
| C | Nil | Nil | Nil | Nil |
| **Tris buffer** | | | | |
| A | 35.28±1.3 | 36.0±1.0 | 28.16±1.8 | 20.26±3.6 |
| B | Retained | Retained | Retained | Retained |
| C | Nil | Nil | Nil | Nil |
| **Tris buffer + 0.1% glucose** | | | | |
| A | 74.85±1.6 | 93.80±1.1 | 53.40±2.01 | 49.28±0.9 |
| B | Retained | Retained | Retained | Retained |
| C | Nil | Nil | Nil | Nil |
| **Half strength PYEG** | | | | |
| A | 100.0±0.0 | 100.0±0.0 | 78.96±0.02 | 62.06±1.68 |
| B | Retained, cells leached | Retained, cells leached | Retained, cells leached | Retained, cells leached |
| C | 6.42±0.26 | 6.26±0.26 | 6.02±0.26 | 6.16±0.2 |
| **V. B. broth** | | | | |
| A | - | - | 51.06±0.45 | 52.6 ±0.6 |
| B | Disintrigated after 2 h | Disintrigated after 2 h | Disintrigated | Disintrigated |
| C | 8.48±0.02 | 8.28±0.08 | 8.28±0.06 | 8.28±0.06 |

A: Cr(VI) reduction (%), residual Cr(VI) was measured by 1,5-diphenyl carbazide method (Park *et al*. 2000) after 24 h of incubation, B: Bead integrity, recorded visually, C: Cell leached in the Cr(VI) reduction medium (log no. of cells $mL^{-1}$ determined by dilution and plating method), Results represent mean of triplicate sets±standard error

## DISCUSSION

Actinomycetes constitute an important group of bacteria that make up nearly 50% of the soil microbial population. In general, actinomycetes are metabolically and biosynthetically versatile, display high resistance to metals and antibiotics and recent reports have confirmed their tolerance as well as capacity to reduce Cr(VI) (Polti *et al*., 2010; Poopal and Laxman, 2008; Elangovan *et al*., 2010).

The bacterial isolate SUK 1205, obtained from metalliferous chromite mine overburden of Odisha, India was identified as *Arthrobacter* sp. SUK 1205 (MTCC 8731 Gen Bank accession No. JQ312666) showed resistance to heavy metal including Cr and developed the property of reducing hexavalent chromium as an adaptive feature in withstanding the toxic mining environment (Dey and Paul, 2013a). *Arthrobacter* sp. SUK 1205 was found to reduce nearly 64% of 2 mM Cr(VI) in V. B. broth after 8 days of incubation (Fig. 1).

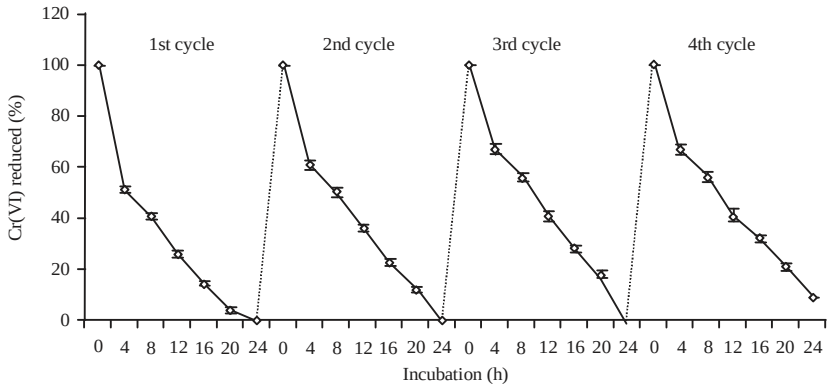


Fig. 7: Recycling of Ca-alginate immobilized cells of *Arthrobacter* sp. SUK 1205 for chromate reduction in batch operations

The V. B. broth, being a synthetic medium contains low level of organic substances and thereby minimized the possible complexation of Cr(VI) with medium constituents and the Cr(VI) reducing capability of the isolate appears to be the accurate reflection of the chromate reducing potential of the isolate SUK 1205. This corroborates the findings of Megharaj *et al*. (2003), where Cr(VI) reduction was assessed using synthetic media. Camargo *et al*. (2004) reported that both intact cells and the cell-free extract of *Arthrobacter crystallopoietes* ES 32 displayed considerable reduction of Cr(VI) to Cr(III). Chromate reduction by SUK 1205 during growth resulted in the formation of characteristics extracellular pale green precipitates. Dey and Paul (2012a) reported the formation of similar green extracellular precipitate by *Arthrobacter* sp. SUK 1201 during growth, while similar blue precipitate formation was also evident during Cr(VI) reduction by *Achromobacter* (Zhu *et al*., 2008a) and *Leucobacter* sp. (Zhu *et al*., 2008b).

With increase in Cr(VI) concentration in the medium, chromate reduction rate decreased significantly, which may possibly be due to the toxicity and reduction in cell number of SUK 1205 (Fig. 2). Similar phenomenon was also evident in *Bacillus* sp., *Ochrobactrum intermedium* SDCr-5 and *Ochrobactrum* sp. CSCr-3 (Wang and Xiao, 1995; Sultan and Hasnain, 2007; He *et al*., 2009).

A high cell density ($10^{10}$ cells $mL^{-1}$) of *Arthrobacter* sp. SUK 1205 was essentially required for nearly 90% reduction of 2 mM (VI) (Fig. 3). Similar results were also found with other actinomycetes such as *Arthrobacter* sp. SUK 1201 (Dey and Paul, 2012a) and *Microbacterium* MP 30 (Pattanapipitpaisal *et al*., 2001), where the rate of Cr(VI) reduction was reported to increase with increase in cell concentration ranging from $10^{7}$-$10^{10}$ cells $mL^{-1}$. Likewise, with increase in cell density, the rate of Cr(VI) reduction was also found to increase in *Bacillus sphaericus* AND 303 (Pal and Paul, 2004) and *Lysinibacillus fusiformis* ZC1 (He *et al*., 2011).

As evident from Table 1, *Arthrobacter* sp. SUK 1205 was able to utilize a variety of organic compounds including low molecular weight carbohydrates, amino acids and fatty acids as source of electron donor for Cr(VI) reduction. Glucose has been reported to be an ideal electron donor for aerobic Cr(VI) reduction by several bacterial spp. such as *Pseudomonas* sp. (Mc Lean and Beveridge, 2001), *Bacillus* sp. (Pal and Paul, 2004), *Bacillus* sp. FM1 (Masood and Mallik, 2011), *Ochrobactrum* sp. (He *et al*., 2009). Likewise, glycerol, glucose and peptone served as efficient electron donors for Cr(VI) reduction by *Arthrobacter* sp. SUK 1201 (Dey and Paul, 2012a).

Temperature and pH were found to have a prominent influence on the chromate reducing potential as well as growth of isolate SUK 1205 (Fig. 4a, b). *Arthrobacter* sp. SUK 1205 effectively reduced chromite with an optimum of pH 7.0. Thacker and Madamwar (2005) and Camargo *et al*. (2003) reported that maximum growth and chromate reduction by *Ochrobactrum* sp. and *Bacillus* sp. ES 29, respectively occurred at pH 7.0. However, Masood and Malik (2011) reported the optimum pH for Cr(VI) reduction by *Bacillus* sp. to be 8. The optimum temperature for chromate reduction by SUK 1205 was found to be 35°C characteristic of mesophilic organism. Elangovan *et al*. (2010) reported maximum chromium reductase activity at 30°C for a pH of 6 in *Arthrobacter rhombi*. Similar optimum temperature for Cr(VI) reduction was also reported in *Arthrobacter* sp. SUK 1201 (Dey and Paul, 2012a), *Ochrobactrum intermedium* SDCr-5 (Sultan and Hasnain, 2007) and in *Bacillus* sp. FM 1 (Malik and Masod, 2011). Optimum temperature for growth and Cr(VI) reduction was found to range

between 35 and 40°C for *Staphylococcus aureus* and *Pediococcus pentosaceus* (Ilias *et al*., 2011), *Ochrobactrum intermedium* Rb-2 (Rida *et al*., 2012), *Ochrobactrum* sp. CSCr-3 (He *et al*., 2009), *Ochrobactrum intermedium* SDCr-5 (Sultan and Hasnain, 2007) and *Nesterenkonia* sp. MF2 (Amoozegar, 2007).

Results in Fig. 5 however, showed that Cr(VI) reducing capability of the isolate was enhanced when Cu(II) and Mn(II) was present in the medium along with Cr(VI). Stimulatory effect of Cu(II), on Cr(VI) reduction activity has been also reported for Cr(VI)-reduction by *Bacillus* sp. ES 29 (Camargo *et al*., 2003), *Ochrobactrum intermedium* strain SDCr-5 (Sultan and Hasnain, 2007), *Ochrobactrum* sp. strain CSCr-3 (He *et al*., 2009), *Amphibacillus* sp. KSUCr3, *Bacillus* sp. KSUCr9a (Ibrahim *et al*., 2011a, b) and *Arthrobacter* sp. SUK 1201 (Dey and Paul, 2012a).

Chromate reductase activity of SUK 1205 was severely affected by the metabolic inhibitors like CCCP, NaF and DCC due to disruption of chemiosmotic gradient, disruption of enolase activity and inhibition of ATPase activity respectively (Table 2). Similar inhibition of Cr(VI) reduction was also sevident with *Arthrobacter* sp. SUK 1201 (Dey and Paul, 2012b), *Stenotrophomonas maltophilia*, *Staphylococcus gallinarum*, *Pantoea* sp. and *Aeromonas* sp. in presence of $NaN_3$ (Alam and Ahmad, 2011). However, Camargo *et al*. (2004) reported that Cr(VI) reduction by *Arthrobacter crystallopoietes* ES 32 was not inhibited by 1 mM cyanide (NaCN) and azide ($NaN_3$). Similarly in other *Streptomyces* species, sodium azide did not inhibit chromium reduction (Das and Chandra, 1990), but a partial inhibition was recorded in *Streptomyces griseus* at a concentration of 1 mM (Laxman and Moore, 2002). The DNP, the uncoupler might have accelerated the respiratory chain linked electron transport mechanism (Wani *et al*., 2007) and thereby showed chromate reductase activity more or less similar to control. Enhancement of Cr(VI) reduction by DNP has also been reported in *Burkholderia cepacia* (Wani *et al*., 2007) and *Staphylococcus gallinarum* (Alam and Ahmad, 2011).

Among the immobilizing matrices tested Ca-alginate beads were most effective which indicate high affinity of Ca ions towards alginate leading to the formation of stronger and stable gel beads with better cellular activities (Cassidy *et al*., 1996; Ganguli and Tripathi, 2002; Camargo *et al*., 2004). Ba-alginate, on the other hand was found to be more sensitive towards different chelating agents such as phosphate, sodium and magnesium ions. The low Cr(VI) reduction by PVA beads might be due to constrains related to diffusion of chromate and electron donor into the beads as demonstrated by Pattanapipitpaisal *et al*. (2001) and Dey and Paul (2014). The MS medium was the most effective one for reduction studies (Table 3) as it minimizes the possible complexation of Cr(VI) with medium components and allow the assessment of Cr(VI) reduction potential more accurately (Megharaj *et al*., 2003; Pal *et al*., 2013; Dey and Paul, 2014). The susceptibility of beads to disintegration in V. B. broth might be due to high sodium ions sufficient enough to depolymerize beads rapidly (Bang and Pazirandeh, 1999).

It was also evident that Ca-alginate beads containing *Arthrobacter* sp. SUK 1205 cells could also be reused to reduce hexavalent chromium at least for four consecutive cycles (Fig. 7) without reduction of chromium reductase activity of the cells and therefore, could be an effective tool for long term bioremediation of chromium pollutants, particularly in aquatic environment.

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